

DOI: <https://doi.org/10.17816/mechnikov71585>

诊断对 *Aspergillus* spp. 敏感的支气管哮喘的新机会

© Yana I. Kozlova, Alexandra E. Uchevatkina, Larisa V. Filippova, Oleg V. Aak,
Valeriy D. Kuznetsov, Ekaterina V. Frolova, Natalia V. Vasilyeva, Nikolay N. Klimko

North-Western State Medical University named after I.I. Mechnikov, Saint Petersburg, Russia

论证。对 *Aspergillus* spp. 敏感的支气管哮喘的诊断越来越重要, 由于疾病的严重, 不受控制的过程以及形成过敏性支气管肺曲霉病的可能性。

研究的目的是评估使用流式细胞术的嗜碱性粒细胞活化试验诊断 *Aspergillus* 敏感的哮喘的可能性。支气管。

材料与方法。对118名过敏性支气管哮喘患者进行了检查。通过酶免疫分析法测定血清中总免疫球蛋白E (IgE) 和对空气过敏原的特异性IgE的水平。嗜碱性粒细胞活化通过流式细胞术使用过敏性试剂盒 (Cellular Analysis of Allergy, Beckman-Coulter, 美国) 进行研究。刺激嗜碱性粒细胞使用了过敏原 *Aspergillus fumigatus* (Alkor Bio, 俄罗斯)。

结果。第一组由57名支气管哮喘患者组成对 *Aspergillus* spp. 不致敏。第二组包括36名支气管哮喘患者对 *Aspergillus* spp. 敏感。第三组由25名过敏性支气管肺曲霉病患者组成。对 *Aspergillus* spp. 致敏的支气管哮喘患者和过敏性支气管肺曲霉病患者, 被过敏原 *Aspergillus fumigatus* 激活的嗜碱性粒细胞数量明显高于支气管哮喘患者组, 达到8.1 [5.2; 20.9]% 和84.6 [75.7; 94.0]%, 分别为 ($p < 0.001$)。研究组的刺激指数从0.7到72.6不等。识别对 *Aspergillus* spp. 敏感的支气管哮喘患者的最佳诊断点 (cut off) 是刺激指数超过2.4, 而对于过敏性支气管肺曲霉病患者则为15.95。在所有对 *Aspergillus* spp. 过敏的患者中在特定IgE水平与 *Aspergillus* spp. 之间建立了正相关。由过敏原 *Aspergillus fumigatus* ($r = 0.792$, $p < 0.001$) 和刺激指数 ($r = 0.796$, $p < 0.05$) 激活的嗜碱性粒细胞比例。

结论。嗜碱性粒细胞活化试验可用作诊断对 *Aspergillus* spp. 过敏的支气管哮喘的附加方法。皮肤试验和特定IgE的结果相互矛盾或阴性的情况下, 以及在缺乏研究 *in vivo* 的可能性的情况下, 该测试对于确认真菌致敏是必要的。

关键词: *Aspergillus* spp.; 哮喘; 嗜碱性粒细胞活化试验。

引用本文:

Kozlova Yal, Uchevatkina AE, Filippova LV, Aak OV, Kuznetsov VD, Frolova EV, Vasilyeva NV, Klimko NN. 诊断对 *Aspergillus* spp. 敏感的支气管哮喘的新机会. *Herald of North-Western State Medical University named after I.I. Mechnikov*. 2021;13(2):67-76. DOI: <https://doi.org/10.17816/mechnikov71585>

DOI: <https://doi.org/10.17816/mechnikov71585>

New opportunities in the diagnosis of asthma with sensitization to *Aspergillus* spp.

© Yana I. Kozlova, Alexandra E. Uchevatkina, Larisa V. Filippova, Oleg V. Aak, Valeriy D. Kuznetsov, Ekaterina V. Frolova, Natalia V. Vasilyeva, Nikolay N. Klimko

North-Western State Medical University named after I.I. Mechnikov, Saint Petersburg, Russia

BACKGROUND: Diagnosis of asthma with sensitization to *Aspergillus* spp. is becoming increasingly important due to the severe, uncontrolled course of the disease and the possibility of the formation of allergic bronchopulmonary aspergillosis.

AIM: To evaluate the possibility of using the basophil activation test using flow cytometry for the diagnosis of asthma with sensitization to *Aspergillus* spp.

MATERIALS AND METHODS: 118 patients with asthma were examined. The levels of total IgE and specific IgE to aero-allergens were determined in the blood serum by the enzyme immunoassay. Basophil activation was studied by flow cytometry using the Allergenicity kit (Cellular Analysis of Allergy, Beckman-Coulter, USA). The allergen *Aspergillus fumigatus* (Alcor Bio, Russia) was used to stimulate basophils.

RESULTS: The first group consisted of 57 patients with asthma without sensitization to *Aspergillus* spp. The second group included 36 patients with asthma with sensitization to *Aspergillus* spp. The third group consisted of 25 patients with allergic bronchopulmonary aspergillosis. The number of basophils activated by the *Aspergillus fumigatus* allergen in patients with asthma with sensitization to *Aspergillus* spp. and allergic bronchopulmonary aspergillosis was significantly higher than in the asthma group and amounted to 8.1 [5.2; 20.9]% and 84.6 [75.7; 94.0]%, respectively ($p < 0.001$). The stimulation index in the study groups ranged from 0.7 to 72.6. The optimal diagnostic point (cut off) for identifying patients with asthma with *Aspergillus* spp. sensitization there was an stimulation index value of more than 2.4, and for patients with allergic bronchopulmonary aspergillosis — 15.95. Among all patients with sensitization to *Aspergillus* spp. a positive correlation was established between the level of specific IgE to *Aspergillus* spp. and the percentage of basophils activated by the allergen *Aspergillus fumigatus* ($r = 0.792$, $p < 0.001$) and stimulation index ($r = 0.796$, $p < 0.05$).

CONCLUSIONS: The basophil activation test can be used as an additional diagnostic method for asthma with sensitization to *Aspergillus* spp. and allergic bronchopulmonary aspergillosis.

Keywords: *Aspergillus* spp.; asthma; basophil activation test.

To cite this article:

Kozlova YaI, Uchevatkina AE, Filippova LV, Aak OV, Kuznetsov VD, Frolova EV, Vasilyeva NV, Klimko NN. New opportunities in the diagnosis of asthma with sensitization to *Aspergillus* spp. *Herald of North-Western State Medical University named after I.I. Mechnikov*. 2021;13(2):67–76. DOI: <https://doi.org/10.17816/mechnikov71585>

DOI: <https://doi.org/10.17816/mechnikov71585>

Новые возможности в диагностике бронхиальной астмы с сенсibilизацией к *Aspergillus spp.*

© Я.И. Козлова, А.Е. Учеваткина, Л.В. Филиппова, О.В. Аак, В.Д. Кузнецов, Е.В. Фролова, Н.В. Васильева, Н.Н. Клишко

Северо-Западный государственный медицинский университет им. И.И. Мечникова, Санкт-Петербург, Россия

Обоснование. Диагностика бронхиальной астмы с сенсibilизацией к *Aspergillus spp.* приобретает все большее значение в связи с тяжелым, неконтролируемым течением заболевания и возможностью формирования аллергического бронхолегочного аспергиллеза.

Цель исследования — оценить возможность применения теста активации базофилов с использованием проточной цитометрии для диагностики бронхиальной астмы с сенсibilизацией к *Aspergillus spp.*

Материалы и методы. Проведено обследование 118 больных аллергической бронхиальной астмой. Уровни общего иммуноглобулина Е (IgE) и специфических IgE к аэроаллергенам определяли в сыворотке крови иммуноферментным методом. Активацию базофилов изучали методом проточной цитометрии с помощью набора Allerginity kit (Cellular Analysis of Allergy, Beckman-Coulter, США). Для стимуляции базофилов в работе использовали аллерген *Aspergillus fumigatus* («Алкор Био», Россия).

Результаты. Первую группу составили 57 больных бронхиальной астмой без сенсibilизации к *Aspergillus spp.* Во вторую группу вошли 36 больных бронхиальной астмой с сенсibilизацией к *Aspergillus spp.* Третью группу составили 25 больных аллергическим бронхолегочным аспергиллезом. Количество базофилов, активированных аллергеном *Aspergillus fumigatus*, у больных бронхиальной астмой с сенсibilизацией к *Aspergillus spp.* и аллергическим бронхолегочным аспергиллезом было достоверно больше, чем в группе пациентов с бронхиальной астмой, и составило 8,1 [5,2; 20,9] % и 84,6 [75,7; 94,0] % соответственно ($p < 0,001$). Индекс стимуляции в исследуемых группах варьировал от 0,7 до 72,6. Оптимальной диагностической точкой (cut off) для выявления больных бронхиальной астмой с сенсibilизацией к *Aspergillus spp.* было значение индекса стимуляции более 2,4, а для больных аллергическим бронхолегочным аспергиллезом — 15,95. Среди всех больных с сенсibilизацией к *Aspergillus spp.* установлена положительная корреляционная связь уровня специфических IgE к *Aspergillus spp.* с долей активированных аллергеном *Aspergillus fumigatus* базофилов ($r = 0,792$, $p < 0,001$) и индексом стимуляции ($r = 0,796$, $p < 0,05$).

Заключение. Тест активации базофилов может быть использован в качестве дополнительного метода диагностики бронхиальной астмы с сенсibilизацией к *Aspergillus spp.* и аллергического бронхолегочного аспергиллеза. Тест необходим для подтверждения микогенной сенсibilизации в случаях противоречивых или отрицательных результатов кожных тестов и специфических IgE, а также при отсутствии возможности проведения исследований *in vivo*.

Ключевые слова: *Aspergillus spp.*; астма; тест активации базофилов.

Как цитировать:

Козлова Я.И., Учеваткина А.Е., Филиппова Л.В., Аак О.В., Кузнецов В.Д., Фролова Е.В., Васильева Н.В., Клишко Н.Н. Новые возможности в диагностике бронхиальной астмы с сенсibilизацией к *Aspergillus spp.* // Вестник Северо-Западного государственного медицинского университета им. И.И. Мечникова. 2021. Т. 13. № 2. С. 67–76. DOI: <https://doi.org/10.17816/mechnikov71585>

论证

支气管哮喘 (BA) 是成年人中最常见的呼吸系统疾病之一, 具有较高的社会经济意义。目前全世界都注意到这种疾病的严重形式的患病率有增加的趋势[1]。在严重支气管哮喘的结构中对 *Aspergillus* spp. 敏感的支气管哮喘[2, 3]。

根据一些研究的结果, 尽管吸入了最大剂量的糖皮质激素, 但仍有多达50%的哮喘控制不足的成年人对 *Aspergillus* spp. 过敏[4]。对霉菌在BA发病机制中的重要作用的认识导致了“真菌致敏的严重BA”一词的出现 (severe asthma with fungal sensitisation, SAFS)。这是一组BA患者, 病程不受控制, 对真菌抗原敏感, 无支气管扩张, 粘液积聚, 总IgE水平低于1000 IU/毫升[5]。

根据计算数据, 真菌致敏的重度BA患者在全世界可达650万人在俄罗斯联邦可达23.1万人[6, 7]。

此外, 对 *Aspergillus* spp. 敏感的BA患者有患过敏性支气管肺曲霉病 (ABPA) 等严重慢性肺部疾病的风险。据专家称, 虽然ABPA影响世界上大约500万人, 但这种疾病往往未被及时发现[6]。ABPA的进展导致患者出现纤维化、呼吸衰竭和残疾[8, 9]。

确认对 *Aspergillus* spp. 的立即超敏反应使用方法 *in vivo*, 对此有许多禁忌症, 并且结果可能不一致。在这方面近几十年来 *in vivo* 方法受到了很多关注, 其优点是对患者的安全性、特异性和标准化的可能性。

除了对系统糖皮质激素的高需求外, 对 *Aspergillus* spp. 有敏感反应的重BA与频繁的生命威胁和高死亡风险有关[10, 11]。这使得及时诊断这种表型成为现代医学的一个紧迫问题。

该研究的目的是评估使用过敏原 *Aspergillus fumigatus* 激活嗜碱性粒细胞的测试的可能性 *in vitro* 使用流式细胞术用于诊断对 *Aspergillus* spp. 敏感的哮喘。

材料与方法

这项研究包括118名成人过敏性哮喘患者。使用测试系统 (Polignost LLC, 俄罗斯) 和一组生物素化过敏原 (Alkor Bio, 俄罗斯) 通过酶免疫测定法测定血清中总免疫球蛋白E (IgE) 和针对真菌、家庭和表皮过敏原的特异性IgE的水平。

嗜碱性粒细胞活化通过流式细胞术使用过敏性试剂盒 (Cellular Analysis of Allergy, Beckman-Coulter, 美国) 进行研究。嗜碱性粒细胞的水平使用标记物 CD3⁻CRTH2⁺ (CRTH2趋化受体) 进行评估。通过增加刺激后细胞上CD203c的表达 *in vitro* 来确定活化嗜碱性粒细胞的数量。缓冲溶液 (阴性对照) 存在下, 外周全血样本用单克隆抗体 CRTH2-FITC / CD203c-PE / CD3-PC7 的三重混合物染色或IgE单克隆抗体 (阳性对照), 或过敏原 *A. fumigatus* (Alkor

Bio, 俄罗斯) 在黑暗中37°C 15分钟。前的研究已经确定了过敏原的最佳浓度[12]。然后使用Allergenicity kit. 试剂盒中的裂解固定试剂进行红细胞裂解。使用Navios Beckman Coulter流式细胞仪 (美国) 在每个样品中计数至少500个嗜碱性粒细胞。评估了嗜碱性粒细胞的自发激活 - CD3⁻CRTH2⁺CD203c⁺ 细胞与缓冲溶液样品中嗜碱性粒细胞总数的比例, 这使得可以区分静息细胞分子的表达水平与细胞活化状态。计数与抗IgE抗体孵育后活化的嗜碱性粒细胞的数量是必要的, 以确认嗜碱性粒细胞的非特异性活化能力, 以排除假阴性反应并增加方法的特异性。

诊断是根据“支气管哮喘治疗和预防全球战略”中提出的建议做出的 (Global Initiative for Asthma, GINA, 2020) [1]。为了检测真菌致敏作用, 使用了国际人类和动物真菌学学会 (International Society for Human and Animal Mycology, ISHAM) 专家提出的标准: 皮肤点刺试验阳性 (≥ 3 毫米) 和/或确定对真菌过敏原的特定IgE水平对应于I类及以上的血清 (≥ 0.35 IU/毫升)。ABPA的诊断是根据R. Agarwal和合著者标准建立的(2013) [13]。

研究期间获得的数据使用STATISTICA 10软件系统和SPSS Statistics 23进行处理。数据以中位数和下四分位数和上四分位数表示 [$Me (Q_1; Q_3)$]。为了评估独立样本之间的差异, 使用了Kruskal-Wallis秩方差分析和非参数Mann-Whitney检验。使用Spearman相关系数评估指标之间的关系。 $p < 0.05$ 为差异有统计学意义。为了评估刺激指数在检测真菌致敏方面的诊断意义, 通过计算ROC曲线下面积 (receiver-operator characteristic) - AUC (area under curve) 进行接受者-操作者特征 (ROC) 分析。这是定量评估之一研究指标的诊断效率。ROC曲线绘制在每个分割点的真阳性 (敏感性) 和假阳性 (特异性) 频率的X轴和Y轴上。用灵敏度和特异性之和的最大值选择分离点的最佳值 (阈值)。

结果与讨论

根据临床和仪器检查结果, 将BA患者分为三组。第一组包括57名对 *Aspergillus* spp. 无致敏的BA患者, 平均年龄为 50 ± 15 岁 (女性-80.7%)。第一组包括36名对 *Aspergillus* spp. 无致敏的BA患者, 平均年龄为 49 ± 14 岁 (女性77.8%)。根据R. Agarwal和合著者确定了25名在BA背景下发生ABPA的患者。第三组患者的平均年龄为 45 ± 16 岁 (女性为64%)。各组在性别和年龄上没有差异。

In vitro 条件中使用流式细胞术所有患者均接受了过敏原 *Aspergillus fumigatus* 的嗜碱性粒细胞活化试验结果见附表。

对 *Aspergillus* spp. 致敏的BA患者和对照组中嗜碱性粒细胞的自发激活率在0.6%到8.3%之间没

表格支气管哮喘患者免疫学检查结果, $n = 118$

Table. Results of immunological examination of patients with asthma, $n = 118$

指标	第一组	第二组	第三组	显著性水平 (p)
	BA ($n = 57$)	BA对 <i>Aspergillus</i> spp. 致敏 ($n = 36$)	ABPA ($n = 25$)	
<i>Aspergillus</i> 异性IgE 水平, IU/毫升	0.02 [0.00; 0.05]	0.90 [0.56; 1.27]	2.20 [1.15; 7.13]	$p_{1-2} < 0.001$ $p_{1-3} < 0.001$ $p_{2-3} < 0.001$
嗜碱性粒细胞的自发 激活, %	2.6 [1.8; 4.4]	2.3 [1.5; 3.1]	2.3 [1.5; 4.3]	$p_{1-2} = 0.12$ $p_{1-3} = 0.59$ $p_{2-3} = 0.45$
IgE-介导的嗜碱性粒 细胞活化, %	71.9 [60.0; 81.7]	74.2 [63.1; 87.3]	74.5 [67.0; 88.1]	$p_{1-2} = 0.34$ $p_{1-3} = 0.21$ $p_{2-3} = 0.72$
激活的嗜碱性粒细胞 数量, %	3.6 [2.3; 5.5]	8.1 [5.2; 20.9]	84.6 [75.7; 94.0]	$p_{1-2} < 0.001$ $p_{1-3} < 0.001$ $p_{2-3} < 0.001$
刺激指数	1.2 [1.0; 1.5]	4.0 [2.5; 11.2]	27.7 [21.1; 48.5]	$p_{1-2} < 0.001$ $p_{1-3} < 0.001$ $p_{2-3} < 0.001$

注意: BA-支气管哮喘; ABPA-过敏性支气管肺曲霉菌病; IgE-免疫球蛋白E。

有差异。IgE介导的嗜碱性粒细胞活化程度在各组之间没有差异, 范围为29.6%至96.9%。介导的嗜碱性粒细胞活化程度在各组之间没有差异, 范围为29.6%至96.9%。

在对*Aspergillus fumigatus*和ABPA过敏的BA患者中, 由*Aspergillus fumigatus*烟曲霉变应原激活的嗜碱性粒细胞数量显著高于BA患者组达到8.1[5.2; 20.9%和84.6[75.7%; 分别为94.0%和94.0% ($p < 0.001$)。

可以接受的是, 不仅通过CD203c标记物的表达响应与过敏原孵育而增加的细胞数量来考虑嗜碱性粒细胞的活化水平, 但通过刺激指数(IS)。IS计算为测试中带有过敏原的活化嗜碱性粒细胞的比例与阴性对照中自发活化的嗜碱性粒细胞的比例之比。IS在对*Aspergillus* spp.敏感的BA患者组中是4.0 [2.5; 11.2]。该指标与对照组有显著差异在BA和ABPA患者的指标之间处于中间位置 ($p < 0.001$)。需要注意的是在ABPA患者组中IS达到的最大值-27.7 [21.1; 48.5]。

Aspergillus spp.对*Aspergillus* spp.敏感的BA患者嗜碱性粒细胞活化试验的直方图示例, 并且对曲霉属不致敏就表示图1。

评估嗜碱性粒细胞活化试验在检测*Aspergillus* spp.致敏性方面的诊断意义, 通过计算曲线下面积进行ROC分析。我们的研究中对*Aspergillus* spp.敏感的患者IS范围为0.7至72.6对*Aspergillus* spp.不敏感的患者为0.7至4.2。AUC

为0.883 (95% CI 0.809-0.956), 敏感性为86.9% (95% CI 76.2-93.2), 特异性为94.7% (95% CI 85.6-98.2) ($p < 0.001$)。这表明该方法具有较高的特异性和灵敏度大于2.4的IS值是检测BA患者真菌致敏的最佳截止点(cut off), 具有高度的可靠性。

在下一阶段在对*Aspergillus*过敏的患者中确定了IS对ABPA诊断的价值。AUC为0.887 (95% CI 0.800-0.972), 敏感性为96.0% (95% CI 80.5-99.3), 特异性为80.6% (95% CI 65.0-90.3) ($p < 0.001$)。因此大于15.95的IS值是识别ABPA患者的最佳截止点(cut off)。

所有对*Aspergillus* spp.过敏的患者中, *Aspergillus* spp.曲霉属的特异性IgE水平之间建立了正相关。由*Aspergillus* spp.过敏原($r = 0.792$, $p < 0.001$)和IS($r = 0.796$, $p < 0.05$)激活的嗜碱性粒细胞的比例。获得的数据证实了嗜碱性粒细胞活化试验与IgE水平对真菌过敏原的标准测定之间的关系, 并BA患者中使其可用于诊断对*Aspergillus* spp.的即时超敏反应。

讨论

确认对*Aspergillus* spp.超敏反应-对*Aspergillus* spp.敏感的重度哮喘患者的一个重要诊断阶段和ABPA。目前已知的实验室方法的选择并不总是能够满足临床医生的需要。应该记住, *Aspergillus* spp.抗原的吸入试验与发生致命性支气管痉挛的风险有关, 不推荐用于临

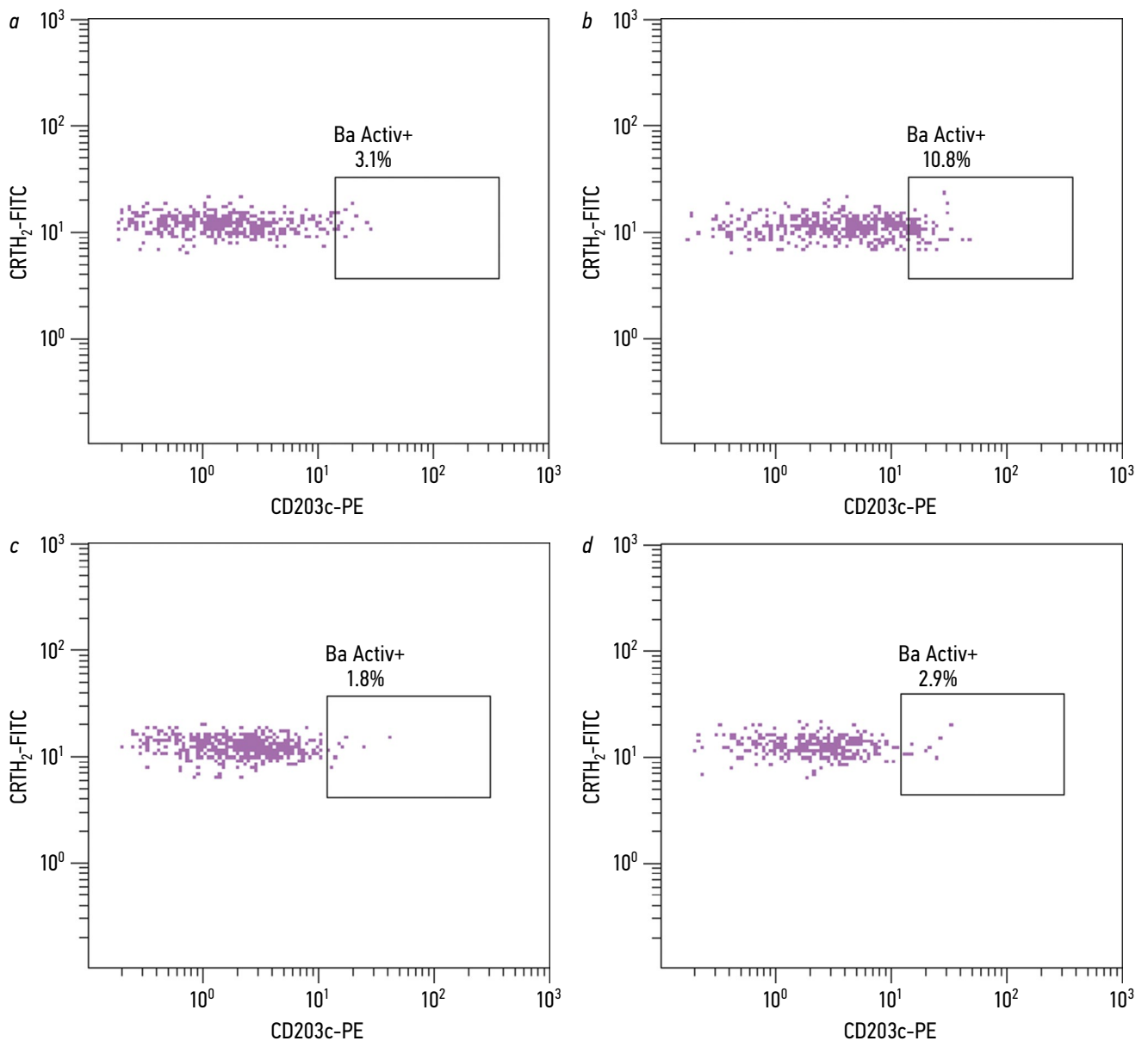


图 1 支气管哮喘患者的嗜碱性粒细胞活化试验。患者C. (a, b) 和患者D. (c, d) 在自发性 (a, c) 后嗜碱性粒细胞的最终门控阶段 ($CD3^-CRTH2^+CD203^{++}$) 和特定的烟曲霉 (b, d) 激活。大部分活化的嗜碱性粒细胞 (b) 确认致敏的存在

Fig. 1. Basophil activation test in patients with asthma. Patients C. (a, b) and patients D. (c, d) at the final basophil gating ($CD3^-CRTH2^+CD203^{++}$) after the spontaneous (a, c) and specific *Aspergillus fumigatus* (b, d) activation. High percentage of the activated basophils (b) confirms the sensibility

床实践[13, 14]。虽然有许多激发试验和皮肤试验的禁忌症, *in vitro* 诊断方法具有特别的相关性[15]。然而, 在不同的实验室中获得的结果并不总是可靠和可重复的。众所周知, IgE的水平在所有诊断算法中都是确定的, 其特征是血清中的含量微不足道。此外, 这类免疫球蛋白可能不存在于循环中, 但在靶细胞嗜碱性嗜酸性粒细胞和肥大细胞上是固定的[16]。

在体外 *in vitro* 过敏最有前途的方向之一是通过流式细胞测定法[17-19]检测特定过敏原对嗜碱性菌的激活。测试的优点是增加了患者的读数、安全性和标准化能力。

嗜碱性杆菌在免疫调节和过敏反应中的作用近年来被高估。通过细胞因子分泌和抗原表达刺激TH2细胞分化的细胞, 参与IGE介导的慢性过敏性炎症的发展, 并在IGE介导的系统性过敏中发挥关键作用[20]。

除了外周血嗜碱性细胞和组织肥大细胞在IGE介导的过敏反应中代表原效应细胞外, 它们也可能参与其他类型的过敏和非过敏反应, 这些反应是基于其他反应机制(补体激活, 非IGE介导刺激和直接细胞毒性作用)。因此, 研究嗜碱性杆菌的功能活性具有重要的诊断意义[21]。

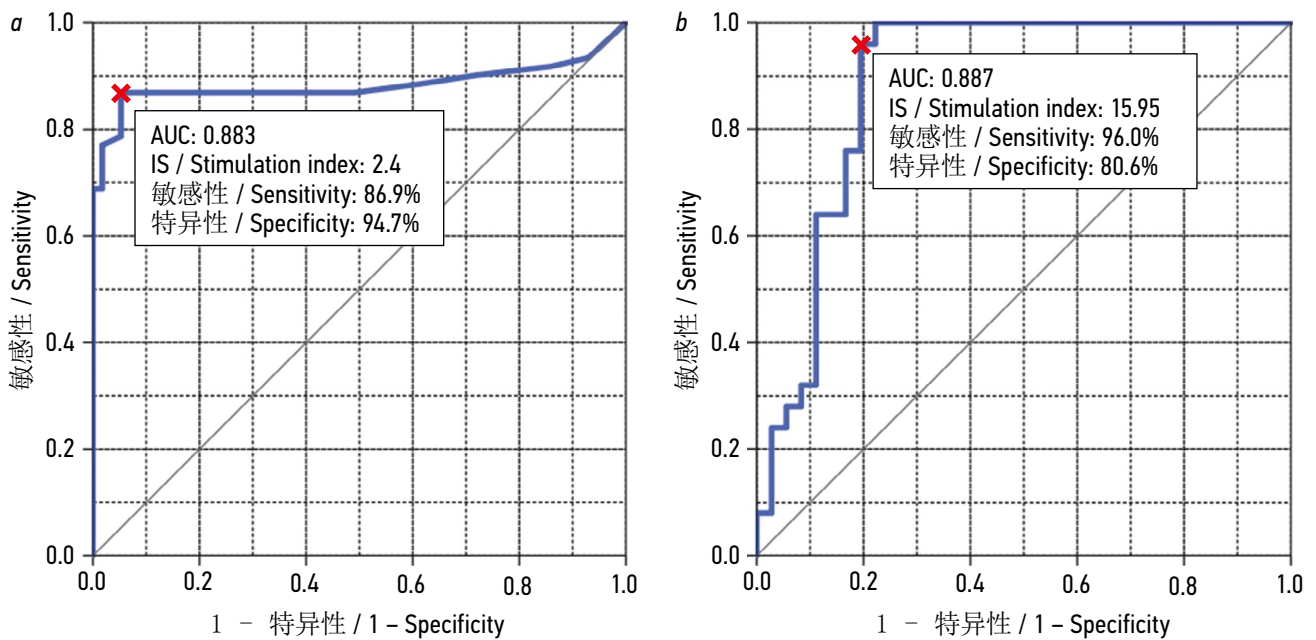


图2 检测对*Aspergillus* spp. 的致敏性在患有支气管哮喘(a)和过敏性支气管肺曲霉病(b)的患者中, ROC曲线说明了最佳截止点(“cut off”)刺激指数(IS)

Fig. 2. ROC curves illustrating the optimal cut off points of the index stimulation to detect sensitization to *Aspergillus* spp. patients with asthma (a) and ABPA (b)

嗜碱性菌活化试验的原理是,当过敏原与固定在嗜碱性菌上的IgE分子接触时,启动酶反应级联,导致脱粒和细胞表面出现额外的分子。目前在过敏诊断中研究最多和最希望的嗜碱性粒细胞活化标志物是CD63和CD203c[22-24]。嗜碱性粒细胞的鉴定以及活化和脱粒标志物表达的评估是使用流式细胞仪进行的。

我们的研究中使用了CD203c标记物(神经细胞表面分化抗原, E-NPP3)。它是一种糖基化II型跨膜分子,属于焦磷酸酶/磷酸二酯酶胞外核苷酸家族——催化寡核苷酸、磷酸酶核苷和NAD水解的酶。造血细胞中,表面E-NPP3仅存在于嗜碱性粒细胞上[25]。少量时,它取决于静息细胞。细胞激活后,CD203c的水平增加了350%[26]。

因此,使用流式细胞术检测嗜碱性粒细胞的活化是一种经济实惠且有前景的即时超敏反应实验室诊断方法。目前,国内外学者已经发表了嗜碱性粒细胞活化试验在昆虫、食物、花粉、药物过敏、慢性荨麻疹等疾病诊断中的应用数据[17-19, 27, 28]。嗜碱性粒细胞活化试验对真菌性过敏患者特别有用,因为目前用于皮肤试验的诊断性真菌过敏原尚未在俄罗斯联邦注册。

确定对*Aspergillus* spp. 的致敏性的研究,借助嗜碱性粒细胞活化的测试很少,并在囊性纤维化患者中进行。根据Gernez和合著者嗜碱性粒细胞活化试验可以区分这组患者的气道定植和对*Aspergillus* 的致敏。已发表的许多著作中,嗜碱性粒细胞活化试验结合*Aspergillus*特异性

IgE和总IgE的测定有助于及时检测囊性纤维化患者的ABPA[29-31]。这些数据与我们之前的研究结果一致[12]。

在本研究过程中获得的结果表明,该测试可用作诊断对*Aspergillus* spp.敏感的BA的附加方法和ABPA。皮肤测试和特定IgE的结果相互矛盾或阴性的情况下,以及在测试可能性的情况下*in vivo*可以进行该测试以确认真菌致敏。

该方法的一个重要优点是使用过敏原*Aspergillus fumigatus*激活嗜碱性粒细胞的测试可在不到2小时内完成;它需要少量全外周血。这可以在用于其他免疫学研究的同一血样中完成,这显著减少了患者的不适。此外,获得定量结果使得使用嗜碱性粒细胞活化试验作为鉴别诊断对*Aspergillus* spp.和ABPA致敏的BA的工具成为可能。及时检测这两种与*Aspergillus* spp.致敏相关的呼吸道疾病,对确定进一步的治疗策略非常重要。

结论

1. 对*Aspergillus* spp.敏感的BA患者中使用嗜碱性粒细胞活化试验证实了真菌致敏。
2. 用于检测对*Aspergillus* spp.敏感的AD的IS阈值为2.4,对于ABLA-15.95。
3. 如果皮肤试验和特定IgE的结果相互矛盾或为阴性,以及无法进行研究*in vivo*时,可以考虑使用嗜碱性粒细胞活化试验来确认真菌致敏。

利益冲突。作者没有利益冲突。

REFERENCES

1. Ginasthma.org [Internet]. The Global Strategy for Asthma Management and Prevention, Global Initiative for Asthma (GINA) 2020. Available from: <http://www.ginasthma.org/>. Accessed: 19.05.2020.
2. Agarwal R. Severe asthma with fungal sensitization. *Curr Allergy Asthma Rep.* 2011;11(5):403–413. DOI: 10.1007/s11882-011-0217-4
3. Rick E, Woolnough K, Pashley C, Wardlaw AJ. Allergic fungal airway disease. *J Investig Allergol Clin Immunol.* 2016;26(6):344–354. DOI: 10.18176/jiaci.0122
4. Agarwal R, Nath A, Aggarwal AN, et al. *Aspergillus* hypersensitivity and allergic bronchopulmonary aspergillosis in patients with acute severe asthma in a respiratory intensive care unit in North India. *Mycoses.* 2010;53(2):138–143. DOI: 10.1111/j.1439-0507.2008.01680x
5. Denning D, O'Driscoll B, Hogaboam C, et al. The link between fungi and asthma: A summary of the evidence. *Eur Respir J.* 2006;27(3):615–626. DOI: 10.1183/09031936.06.00074705
6. Denning DW, Pleuvry A, Cole DC. Global burden of allergic bronchopulmonary aspergillosis with asthma and its complication chronic pulmonary aspergillosis in adults. *Med Mycol.* 2013;51(4):361–370. DOI: 10.3109/13693786.2012.738312
7. Klimko NN, Kozlova YI, Khostelidi SN, et al. The prevalence of serious and chronic fungal diseases in Russian Federation on LIFE program model. *Problems in medical mycology.* 2014;16(1):3–9. (In Russ.)
8. Kosmidis C, Denning D. The clinical spectrum of pulmonary aspergillosis. *Thorax.* 2015;70(3):270–277. DOI: 10.1136/thoraxjnl.2014.206291
9. Agarwal R, Aggarwal A, Gupta D, Jindal SK. *Aspergillus* hypersensitivity and allergic bronchopulmonary aspergillosis in patients with bronchial asthma: systematic review and meta-analysis. *Int J Tuberc Lung Dis.* 2009;13(8):936–944.
10. Goh KJ, Yii ACA, Lapperre TS, et al. Sensitization to *Aspergillus* species is associated with frequent exacerbations in severe asthma. *J Asthma Allergy.* 2017;10:131–140. DOI: 10.2147/JAA.S130459
11. Fairs A, Agbetile J, Hargadon B, et al. IgE sensitization to *Aspergillus fumigatus* is associated with reduced lung function in asthma. *Am J Respir Crit Care Med.* 2010;182(11):1362–1368. DOI: 10.1164/rccm.201001.00870C
12. Kozlova YI, Frolova EV, Uchevatkina AE, et al. Basophile activation test for the diagnostics of fungal sensitization in the patients with cystics fibrosis. *Medical Immunology.* 2019;21(5):919–928. (In Russ.). DOI: 10.15789/1563-0625-2019-5-919-928
13. Agarwal R, Chakrabarti A, Shah A, et al. Allergic bronchopulmonary aspergillosis: review of literature and proposal of new diagnostic and classification criteria. *Clin Exp Allergy.* 2013;43(8):850–873. DOI: 10.1111/cea.12141
14. Agarwal R, Hazarika B, Gupta D, et al. *Aspergillus* hypersensitivity in patients with chronic obstructive pulmonary disease: COPD as a risk factor for ABPA? *Med Micol.* 2010;48(7):988–994. DOI: 10.3109/13693781003743148
15. Oppenheimer J, Nelson HS. Skin testing. *Ann Allergy Asthma Immunol.* 2006;96(2 Suppl 1):S6–12. DOI: 10.1016/S1081-1206(10)60895-2
16. Karasuyama H, Tsujimura Y, Obata K, Mukai K. Role for basophils in systemic anaphylaxis. *Chem Immunol Allergy.* 2010;95:85–87. DOI: 10.1159/000315939
17. Sanz ML, Gamboa PM, De Weck AL. Cellular tests in the diagnosis of drug hypersensitivity. *Curr Pharm Des.* 2008;14(27):2803–2808. DOI: 10.2174/138161208786369722
18. Hausmann OV, Gentinetta T, Bridts CH, Ebo DG. The basophil activation test in immediate-type drug allergy. *Immunol Allergy Clin North Am.* 2009;29(3):555–566. DOI: 10.1016/j.iac.2009.04.011
19. Potapińska O, Górska E, Zawadzka-Krajewska A, et al. The usefulness of CD203c expression measurement on basophils after activation with grass pollen and Dermatophagoides pteronyssinus antigens. Preliminary study. *Pneumonol Alergol Pol.* 2009;77(2):138–144. (In Polish)
20. Knol EF, Mul FP, Jansen H, et al. Monitoring human basophil activation via CD63 monoclonal antibody 435. *J Allergy Clin Immunol.* 1991;88(3 Pt 1):328–338. DOI: 10.1016/0091-6749(91)90094-5
21. Kang MG, Song WJ, Park HK, et al. Basophil activation test with food additives in chronic urticaria patients. *Clin Nutr Res.* 2014;3(1):9–16. DOI: 10.7762/2Fnr.2014.3.1.9
22. Boumiza R, Debard AL, Monneret G. The basophil activation test by flow cytometry: recent development in clinical studies, standardization and emerging perspectives. *Clin Mol Allergy.* 2005;3:9. DOI: 10.1186/1476-7961-3-9
23. Chirumbolo S, Vella A, Ortolani R, et al. Differential response of human basophil activation markers: a multiparameter flow cytometry approach. *Clin Mol Allergy.* 2008;6:12. DOI: 10.1186/1476-7961-6-12
24. Mikkelsen S, Bibby BM, Dolberg MKB, et al. Basophil sensitivity through CD63 or CD203c is a functional measure for specific immunotherapy. *Clin Mol Allergy.* 2010;8(1):2. DOI: 10.1186/1476-7961-8-2
25. Buhring HJ, Seiffert M, Giesert C, et al. The basophil activation marker deined by antibody 97A6 is identical to the ectonucleotide pyrophosphatase/phosphodiesterase 3. *Blood.* 2001;97(10):3303–3305. DOI: 10.1182/blood.v97.10.3303
26. Chirumbolo S, Vella A, Ortolani R, et al. Differential response of human basophil activation markers: a multiparameter flow cytometry approach. *Clin Mol Allergy.* 2008;6:12. DOI: 10.1186/1476-7961-6-12
27. Shabanov DV, Lazarenko LL, Fedoskova TG, Rybnikova EA. Diagnostics of Hymenoptera venom allergy. *Russian medical journal.* 2019;27(3):40–44. (In Russ.)
28. Sinelnikova NA, Bychkova NV, Kalinina NM. Features of immune response and basophil activation in children with chronic urticaria. *Medical Immunology (Russia).* 2015;17(1):39–46. (In Russ.). DOI: 10.15789/1563-0625-2015-1-39-46
29. Gernez Y, Dunn CE, Everson C, et al. Blood basophils from cystic fibrosis patients with allergic bronchopulmonary aspergillosis are primed and hyper-responsive to stimulation by aspergillus allergens. *J Cyst Fibros.* 2012;11(6):502–510. DOI: 10.1016/j.jcf.2012.04.008
30. Gernez Y, Waters J, Mirković B, et al. Blood basophil activation is a reliable biomarker of allergic bronchopulmonary aspergillosis in cystic fibrosis. *Eur Respir J.* 2016;47(1):177–185. DOI: 10.1183/13993003.01068-2015
31. Mircovic B, Lavelle GM, Azim AA, et al. The basophil surface marker CD203C identifies *Aspergillus* species sensitization in patients with cystic fibrosis. *J Allergy Clin Immunol.* 2016;137(2):436–443. DOI: 10.1016/j.jaci.2015.07.045

СПИСОК ЛИТЕРАТУРЫ

1. Ginasthma.org [Электронный ресурс]. The Global Strategy for Asthma Management and Prevention, Global Initiative for Asthma (GINA) 2020. Режим доступа: <http://www.ginasthma.org/>. Дата обращения: 19.05.2020.
2. Agarwal R. Severe asthma with fungal sensitization // *Curr. Allergy. Asthma Rep.* 2011. Vol. 11, No. 5. P. 403–413. DOI: 10.1007/s11882-011-0217-4
3. Rick E., Woolnough K., Pashley C., Wardlaw A.J. Allergic fungal airway disease // *J. Investig. Allergol. Clin. Immunol.* 2016. Vol. 26, No. 6. P. 344–354. DOI: 10.18176/jiaci.0122
4. Agarwal R., Nath A., Aggarwal A.N. et al. *Aspergillus* hypersensitivity and allergic bronchopulmonary aspergillosis in patients with acute severe asthma in a respiratory intensive care unit in North India // *Mycoses.* 2010. Vol. 53, No. 2. P. 138–143. DOI: 10.1111/j.1439-0507.2008.01680x
5. Denning D., O'Driscoll B., Hogaboam C. et al. The link between fungi and asthma: A summary of the evidence // *Eur. Respir. J.* 2006. Vol. 27, No. 3. P. 615–626. DOI: 10.1183/09031936.06.00074705
6. Denning D.W., Pleuvry A., Cole D.C. Global burden of allergic bronchopulmonary aspergillosis with asthma and its complication chronic pulmonary aspergillosis in adults // *Med. Mycol.* 2013. Vol. 51, No. 4. P. 361–370. DOI: 10.3109/13693786.2012.738312
7. Клишко Н.Н., Козлова Я.И., Хостелиди С.Н. и др. Распространенность тяжелых и хронических микотических заболеваний в Российской Федерации по модели LIFE program // *Проблемы медицинской микологии.* 2014. Т. 16, № 1. С. 3–9.
8. Kosmidis C., Denning D. The clinical spectrum of pulmonary aspergillosis // *Thorax.* 2015. Vol. 70, No. 3. P. 270–277. DOI: 10.1136/thoraxjnl.2014.206291
9. Agarwal R., Aggarwal A., Gupta D., Jindal S.K. *Aspergillus* hypersensitivity and allergic bronchopulmonary aspergillosis in patients with bronchial asthma: systematic review and meta-analysis // *Int. J. Tuberc. Lung Dis.* 2009. Vol. 13, No. 8. P. 936–944.
10. Goh K.J., Yii A.C.A., Lapperre T.S. et al. Sensitization to *Aspergillus* species is associated with frequent exacerbations in severe asthma // *J. Asthma Allergy.* 2017. Vol. 10. P. 131–140. DOI: 10.2147/JAA.S130459
11. Fairs A., Agbetile J., Hargadon B. et al. IgE sensitization to *Aspergillus fumigatus* is associated with reduced lung function in asthma // *Am. J. Respir. Crit. Care Med.* 2010. Vol. 182, No. 11. P. 1362–1368. DOI: 10.1164/rccm.201001.00870C
12. Козлова Я.И., Фролова Е.В., Учеваткина А.Е. и др. Тест активации базофилов для диагностики аллергического бронхолегочного аспергиллеза у больных муковисцидозом // *Медицинская иммунология.* 2019. Т. 21, № 5. С. 919–928. DOI: 10.15789/1563-0625-2019-5-919-928
13. Agarwal R., Chakrabarti A., Shah A. et al. Allergic bronchopulmonary aspergillosis: review of literature and proposal of new diagnostic and classification criteria // *Clin. Exp. Allergy.* 2013. Vol. 43, No. 8. P. 850–873. DOI: 10.1111/cea.12141
14. Agarwal R., Hazarika B., Gupta D. et al. *Aspergillus* hypersensitivity in patients with chronic obstructive pulmonary disease: COPD as a risk factor for ABPA? // *Med. Micol.* 2010. Vol. 48, No. 7. P. 988–994. DOI: 10.3109/13693781003743148
15. Oppenheimer J., Nelson H.S. Skin testing // *Ann. Allergy Asthma Immunol.* 2006. Vol. 96, No. 2 Suppl 1. P. S6–12. DOI: 10.1016/S1081-1206(10)60895-2
16. Karasuyama H., Tsujimura Y., Obata K., Mukai K. Role for basophils in systemic anaphylaxis // *Chem. Immunol. Allergy.* 2010. Vol. 95. P. 85–87. DOI: 10.1159/000315939
17. Sanz M.L., Gamboa P.M., De Weck A.L. Cellular tests in the diagnosis of drug hypersensitivity // *Curr. Pharm. Des.* 2008. Vol. 14, No. 27. P. 2803–2808. DOI: 10.2174/138161208786369722
18. Hausmann O.V., Gentinetta T., Bridts C.H., Ebo D.G. The basophil activation test in immediate-type drug allergy // *Immunol. Allergy Clin. North. Am.* 2009. Vol. 29, No. 3. P. 555–566. DOI: 10.1016/j.iac.2009.04.011
19. Potapińska O., Górska E., Zawadzka-Krajewska A. et al. The usefulness of CD203c expression measurement on basophils after activation with grass pollen and Dermatophagoides pteronyssinus antigens. Preliminary study // *Pneumonol. Alergol. Pol.* 2009. Vol. 77, No. 2. P. 138–144. (In Polish)
20. Knol E.F., Mul F.P., Jansen H. et al. Monitoring human basophil activation via CD63 monoclonal antibody 435 // *J. Allergy Clin. Immunol.* 1991. Vol. 88, No. 3 Pt 1. P. 328–338. DOI: 10.1016/0091-6749(91)90094-5
21. Kang M.G., Song W.J., Park H.K. et al. Basophil activation test with food additives in chronic urticarial patients // *Clin. Nutr. Res.* 2014. Vol. 3, No. 1. P. 9–16. DOI: 10.7762/2.Cnrc.2014.3.1.9
22. Boumiza R., Debard A.L., Monneret G. The basophil activation test by flow cytometry: recent development in clinical studies, standardization and emerging perspectives // *Clin. Mol. Allergy.* 2005. Vol. 3. P. 9. DOI: 10.1186/1476-7961-3-9
23. Chirumbolo S., Vella A., Ortolani R. et al. Differential response of human basophil activation markers: a multiparameter flow cytometry approach // *Clin. Mol. Allergy.* 2008. Vol. 6. P. 12. DOI: 10.1186/1476-7961-6-12
24. Mikkelsen S., Bibby B.M., Dolberg M.K.B. et al. Basophil sensitivity through CD63 or CD203c is a functional measure for specific immunotherapy // *Clin. Mol. Allergy.* 2010. Vol. 8, No. 1. P. 2. DOI: 10.1186/1476-7961-8-2
25. Buhning H.J., Seiffert M., Giesert C. et al. The basophil activation marker defined by antibody 97A6 is identical to the ectonucleotide pyrophosphatase/phosphodiesterase 3 // *Blood.* 2001. Vol. 97, No. 10. P. 3303–3305. DOI: 10.1182/blood.v97.10.3303
26. Chirumbolo S., Vella A., Ortolani R. et al. Differential response of human basophil activation markers: a multiparameter flow cytometry approach // *Clin. Mol. Allergy.* 2008. Vol. 6. P. 12. DOI: 10.1186/1476-7961-6-12
27. Шабанов Д.В., Лазаренко Л.Л., Федоскова Т.Г., Рыбникова Е.А. Особенности диагностики аллергии к яду перепончатокрылых насекомых // *Российский медицинский журнал.* 2019. Т. 27, № 3. С. 40–44.
28. Синельникова Н.А., Бычкова Н.В., Калинина Н.М. Особенности иммунного ответа и активации базофилов у детей с хронической крапивницей // *Медицинская иммунология.* 2015. Т. 17, № 1. С. 39–46. DOI: 10.15789/1563-0625-2015-1-39-46

29. Gernez Y., Dunn C.E., Everson C. et al. Blood basophils from cystic fibrosis patients with allergic bronchopulmonary aspergillosis are primed and hyper-responsive to stimulation by aspergillus allergens // *J. Cyst. Fibros.* 2012. Vol. 11, No. 6. P. 502–510. DOI: 10.1016/j.jcf.2012.04.008

30. Gernez Y., Waters J., Mirković B. et al. Blood basophil activation is a reliable biomarker of allergic bronchopulmonary aspergillosis

in cystic fibrosis // *Eur. Respir. J.* 2016. Vol. 47, No. 1. P. 177–185. DOI: 10.1183/13993003.01068-2015

31. Mircovic B., Lavelle G.M., Azim A.A. et al. The basophil surface marker CD203C identifies *Aspergillus* species sensitization in patients with cystic fibrosis // *J. Allergy Clin. Immunol.* 2016. Vol. 137, No. 2. P. 436–443. DOI: 10.1016/j.jaci.2015.07.045

AUTHORS INFO

***Yana I. Kozlova**, MD, Cand. Sci. (Med.), Assistant Professor;
address: 1/28 Santiago de Cuba str.,
Saint Petersburg, 194291, Russia;
ORCID: <https://orcid.org/0000-0002-4602-2438>;
eLibrary SPIN: 5842-6039;
e-mail: kozlova510@mail.ru

Alexandra E. Uchevatkina, MD, Cand. Sci. (Med.);
eLibrary SPIN: 3001-4022;
e-mail: a.uchevatkina@szgmu.ru

Larisa V. Filippova, MD, Cand. Sci. (Med.);
eLibrary SPIN: 6810-0871;
e-mail: larisa.filippova@szgmu.ru

Oleg V. Aak, Cand. Sci. (Chem.);
eLibrary SPIN: 1198-7810; e-mail: oleg.aak@szgmu.ru

Valeriy D. Kuznetsov, PhD student;
e-mail: valeriy_smith@inbox.ru

Ekaterina V. Frolova, MD, Cand. Sci. (Med.);
eLibrary SPIN: 9904-8776; e-mail: ekaterina.frolova@szgmu.ru

Natalya V. Vasilyeva, Dr. Sci. (Biol.),
Professor, Honoured Science Worker;
eLibrary SPIN: 9215-4069; e-mail: mycobiota@szgmu.ru

Nikolay N. Klimko, MD, Dr. Sci. (Med.), Professor;
e-mail: n_klimko@mail.ru

ОБ АВТОРАХ

***Яна Игоревна Козлова**, канд. мед. наук, доцент;
адрес: Россия, 194291, Санкт-Петербург,
ул. Сантьяго-де-Куба, д. 1/28;
ORCID: <https://orcid.org/0000-0002-4602-2438>;
eLibrary SPIN: 5842-6039;
e-mail: kozlova510@mail.ru

Александра Евгеньевна Учеваткина, канд. мед. наук;
eLibrary SPIN: 3001-4022;
e-mail: a.uchevatkina@szgmu.ru

Лариса Вячеславовна Филиппова, канд. мед. наук;
eLibrary SPIN: 6810-0871;
e-mail: larisa.filippova@szgmu.ru

Олег Владимирович Аак, канд. хим. наук;
eLibrary SPIN: 1198-7810; e-mail: oleg.aak@szgmu.ru

Валерий Дмитриевич Кузнецов, аспирант;
e-mail: valeriy_smith@inbox.ru

Екатерина Васильевна Фролова, канд. мед. наук;
eLibrary SPIN: 9904-8776; e-mail: ekaterina.frolova@szgmu.ru

Наталья Всеволодовна Васильева, д-р биол. наук,
профессор, заслуженный деятель науки РФ;
eLibrary SPIN: 9215-4069; e-mail: mycobiota@szgmu.ru

Николай Николаевич Клишко, д-р мед. наук, профессор;
e-mail: n_klimko@mail.ru